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DOMANDE PROVA ORALE

Busta n. 1 *(sorteggiata)*

1) I virus adenoassociati: descrizione delle loro proprietà.
2) La candidata descriva un suo progetto di ricerca inerente alle tematiche del bando.
3) La candidata illustri alcuni sensori geneticamente codificati per parametri intracellulari.

Busta n. 2 *(non sorteggiata)*

1) Tecniche di introduzione di materiale genetico in cellule e organoidi tramite gli adenovirus e i virus adenoassociati.
2) La candidata descriva un suo progetto di ricerca inerente alle tematiche del bando.
3) La candidata illustri alcuni promotori comunemente usati per i virus adenoassociati.

Il Responsabile del procedimento
Dott.ssa Michela La Ferla

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RESEARCH PAPER

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Mutation of nucleotides around the +1 position of type 3 polymerase III promoters: The effect on transcriptional activity and start site usage

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ABSTRACT

Type 3 RNA polymerase III (Pol III) promoters are widely used for the expression of small RNAs such as short hairpin RNA and guide RNA in the popular RNAi and CRISPR-Cas gene regulation systems. Although it is generally believed that type 3 Pol III promoters use a defined transcription start site (+1 position), most man-made promoter constructs contain local sequence alterations of which the impact on transcription efficiency and initiation accuracy is not known. For three human type 3 Pol III promoters (7SK, U6, and H1), we demonstrated that the nucleotides around the +1 position affect both the transcriptional efficiency and start site selection. Human 7SK and U6 promoters with A or G at the +1 position efficiently produced small RNAs with a precise +1 start site. The human H1 promoter with +1A or G also efficiently produced small RNAs but from multiple start sites in the −3/−1 window. These results provide new insights for the design of vectors for accurate expression of designed small RNAs for research and therapeutic purposes.

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Introduction

Small RNAs play regulatory roles in diverse intracellular processes in both eukaryotes and prokaryotes. In addition, small RNA molecules have been exploited as powerful tools for biomedical research and therapeutic purposes. This includes small interfering RNA (siRNA) or short hairpin RNA (shRNA) in RNA interference (RNAi) experiments, guide RNA (gRNA) in clustered regularly interspaced palindromic repeats (CRISPR)-Cas applications and RNA molecules such as aptamers, ribozymes, and antisense RNA.[1-7] Type 3 polymerase III (Pol III) promoter-based vectors have been developed to express these small RNAs inside cells because of high transcriptional activity and defined transcription initiation and termination sites, thus allowing the production of very precise RNA molecules.

The accurate expression of designed small RNAs is important for the proper and specific execution of their function. For instance, for shRNA molecules, the sequence precision is critical because the stem-loop structure is processed by the Dicer endonuclease that measures ~21-nucleotides (nt) from the 5′ terminus and length variation at this end will yield a different RNA duplex with different silencing activity and specificity.[8,9] The recently described Dicer-independent AgoshRNA molecules are directly loaded into Argonaute 2 (Ago2) for processing and subsequent mRNA silencing,[10] but the identity of the 5′ end nt was shown to be critical for AgoshRNA silencing activity and specificity.[11,12] The prokaryotic CRISPR-Cas9 system has been adapted for gene editing in mammalian cells and requires a gRNA to guide the Cas9 endonuclease for site-specific genome editing.[2,13,14] The synthesis of a precise gRNA molecule is extremely important because the 5′ end 20-nt of the gRNA plays a role in target site recognition.[13,15] The newly discovered Cpf1 endonuclease uses a single ~43-nt CRISPR-derived RNA (crRNA) as guide

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